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Minireview

Molecules and Cells

Protein *N*-Glycosylation, Protein Folding, and Protein Quality Control

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Quality control of protein folding represents a fundamental cellular activity. Early steps of protein *N*-glycosylation involving the removal of three glucose and some specific mannose residues in the endoplasmic reticulum have been recognized as being of importance for protein quality control. Specific oligosaccharide structures resulting from the oligosaccharide processing may represent a glyco-code promoting productive protein folding, whereas others may represent glyco-codes for routing not correctly folded proteins for dislocation from the endoplasmic reticulum to the cytosol and subsequent degradation. Although quality control of protein folding is essential for the proper functioning of cells, it is also the basis for protein folding disorders since the recognition and elimination of non-native conformers can result either in loss-of-function or pathological-gain-of-function. The machinery for protein folding control represents a prime example of an intricate interactome present in a single organelle, the endoplasmic reticulum. Here, current views of mechanisms for the recognition and retention leading to productive protein folding or the eventual elimination of misfolded glycoproteins in yeast and mammalian cells are reviewed.

INTRODUCTION

Fundamental to the normal functioning of a single cell or multicellular organism is control over their entire metabolism. Control is important for the central dogma of molecular biology that DNA leads to RNA, which in turn leads to protein. In this context, control is related to decision-making about gene expression, and control over transcription and translation. All newly made proteins that enter the secretory pathway undergo various post-translational modifications and an important one among them is the process of protein *N*-glycosylation, which commences in the rough endoplasmic reticulum (rER) (Zuber and Roth, 2009). Since protein *N*-glycosylation is genetically determined, it would fit in an extended version of the central dogma that protein leads to glycosylation. In general terms, *N*-glycosylation of proteins is important for the accomplishment of their various biological tasks, which are related to the specific function a particular protein fulfils (Gabius, 2009). On the other hand, *N*-glycosylation confers stability and solubility to proteins, and protects them against proteases. However, *N*-glycosylation, independent of its contributions to a particular function of specific proteins, plays a more general role early in the life of proteins, which is in the quality control of protein folding (Ellgaard and Helenius, 2003; Roth, 2002; Roth et al., 2008). Its role in this process is of decisive importance for the fate of newly synthesized secretory and membrane glycoproteins. The rER is not only the site of synthesis of secretory and membrane proteins but also of initial steps of protein *N*-glycosylation and provides an environment promoting protein folding (Fig. 1). Thus, the rER is also the main organelle in which the quality control of protein folding takes place. Since the pre-Golgi intermediates, which are composed of vesiculo-tubular clusters (Bannykh et al., 1996; Fan et al., 2003), also house quality control machinery proteins (Lucocq et al., 1986; Zuber et al., 2000; 2001), they appear to be involved in this process as well.

This mini-review provides an overview about how protein *N*-glycosylation and protein folding in the rER are interrelated in the fundamental cellular process of quality control of protein folding.

Trimming an oligosaccharide makes for a long story: the generation of glyco-codes

The membrane of the rER is the site of the multistep synthesis of an elaborate oligosaccharide composed of three glucose, nine mannose and two *N*-acetylglucosamine residues (Fig. 2), which is covalently linked to dolichol pyrophosphate (Burda and Aebi, 1999). Both, the biosynthetic process and the resulting oligosaccharide are evolutionary conserved from yeast to mammals. The lipid-linked oligosaccharide is co-translationally transferred *en bloc* to an Asn-sequon (N-X-S/T) present in nascent polypeptides by the multisubunit oligosaccharyltransferase (Chavan and Lennarz, 2006; Kelleher and Gilmore, 2006), which forms a complex with the Sec61 translocon and associated ribosome. As already mentioned for the biosynthesis of

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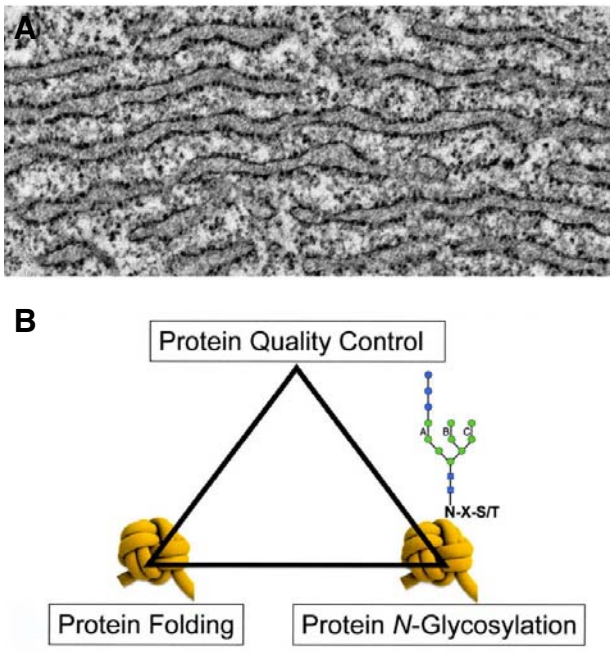

Fig. 1. (A) Electron micrograph showing rough endoplasmic reticulum cisternae of a secretory mammalian cell. (B) Protein quality control, protein folding and protein *N*-glycosylation are related to each other like a Bermuda triangle resulting in the disappearance (by proteasomes or autophagy) of not correctly folded glycoproteins.

the lipid-linked oligosaccharide, the oligosaccharyltransferase-mediated transfer of the oligosaccharide to protein is also an evolutionary conserved process. The subsequently occurring processing reactions on the protein-linked oligosaccharide encompass the sequential removal of all three glucose residues of the branch A (Fig. 3) and some of the mannose residues of the branch B and C, as well as branch A. These so-called trimming reactions have been shown to be conserved, in principle (Table 1), from yeast to higher eukaryotes (Kornfeld and Kornfeld, 1985; Roth, 1987). Although the enzymatic basis for glucose and mannose trimming could be elucidated in great detail, the biological significance of the trimming reactions remained obscure for some time. The core of the puzzle was why such an elaborate structure was made in the first place when it was destined to become quickly trimmed.

The α1, 2-linked outermost glucose residue is removed from the oligosaccharide probably immediately after its transfer to the polypeptide by oligosaccharyltransferase (Fig. 3). The reaction is catalyzed by glucosidase I (Hettkamp et al., 1984; Kalz-Fuller et al., 1995), which is a neutral processing α1, 2 exoglucosidase of the glycosyl hydrolase family 63 with a type II membrane protein topology. Removal of the outermost glucose residue by glucosidase I prevents further interaction of the oligosaccharide with the oligosaccharyltransferase. The two inner α1, 3-linked glucose residues are subsequently removed by glucosidase II (Fig. 3) (Brada and Dubach, 1984; Burns and Touster, 1982). This neutral processing α1, 3 exoglucosidase belongs to the glycosyl hydrolase family 31 and is a luminal glycoprotein. The enzyme is composed of two subunits, a catalytic α subunit and a regulatory β subunit. In addition, the catalytic subunit of glucosidase II, due to alternative splicing, exists in two isoforms (Pelletier et al., 2000; Ziak et al., 2001). The glucose trimming reactions by glucosidase I and II occur equally

Table 1. The various quality control machinery proteins are not ubiquitous.

	S. cerevisiae	*S. pombe*	*D. melanogaster*	Mammalian cells
Glucosidase I	+	+	+	+
Glucosidase II	+	+	+	+
Cnx/Crt*	+/-	+/-	+/+	+/+
Glucosyltransferase	-	+	+	+
ER mannosidase I	+	-	+	+

* Cnx, calnexin; crt, calreticulin

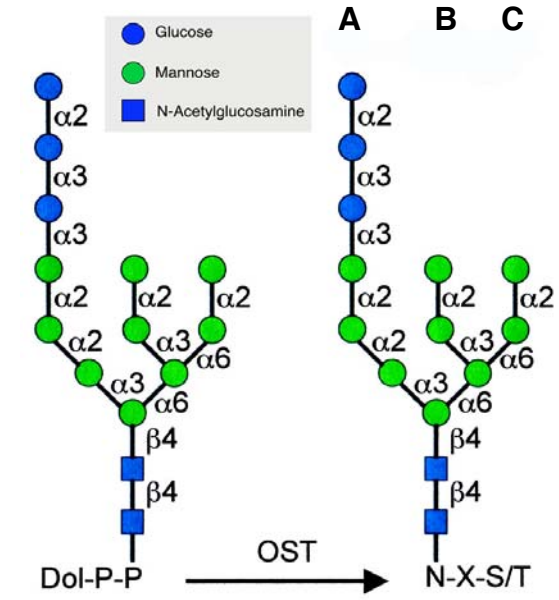

Fig. 2. Schematic presentation of the lipid-linked pre-assembled $Glc_3Man_9GlcNAc_2$ oligosaccharide and its transfer to an Asn sequon by oligosaccharyltransferase (OST). A, B, and C designate the three distinct oligosaccharide branches.

in *Saccharomyces cerevisiae*, *Schizosaccharomyces pompe*, *Drosophila melanogaster* and mammalian cells (Table 1).

As expected from its function, glucosidase II was detected by immunogold electron microscopy throughout the rER, and additionally in the smooth ER and in vesiculo-tubular clusters constituting the pre-Golgi intermediates (Lucocq et al., 1986; Roth et al., 1990; Zuber et al., 2000).

The functional implications of the glucose-trimming reactions in regard to quality control of protein folding could be identified through work in yeast and mammalian cells. Here, an important tool in addition to inhibitors of glucosidases was provided by the cloning of mammalian glucosidase II (Flura et al., 1997), which permitted the identification of an ORF in *S. cerevisiae* coding for the yeast enzyme. This actually provided the basis for the subsequent elucidation of the *in vivo* functional role of glucose-trimmed oligosaccharides. For this, *S. cerevisiae* strains were created that synthesized non-glucosylated, mono-glucosylated or di-glucosylated glycoproteins in the ER (Jakob et al., 1998b). Although no growth phenotype could be observed in yeast strains expressing mono-glucosylated oligosaccharides, a lower degree of the unfolded protein response induction was found under DTT-induced ER stress. Thus, mono-glucosylated oligosaccharides in *S. cerevisiae* represent a positive signal for

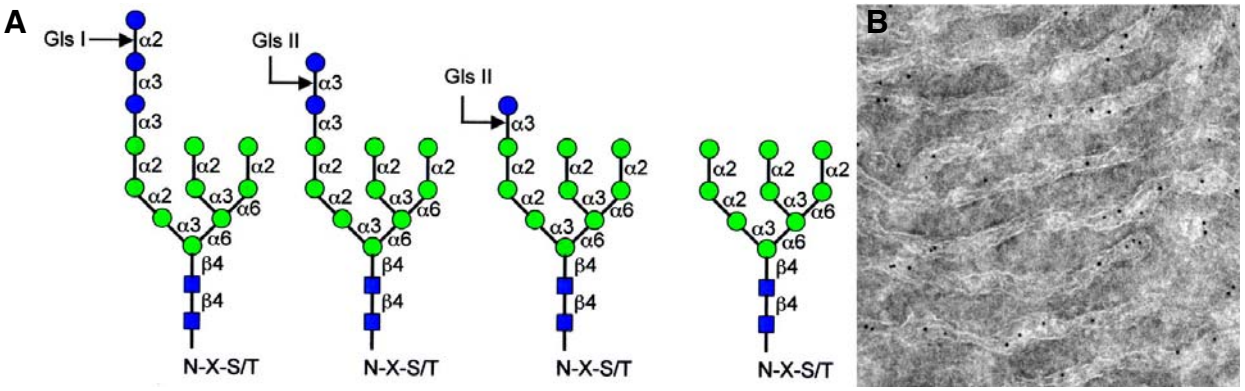

Fig. 3. (A) Schematic presentation of the sequential glucose trimming by glucosidase I (Gls I) and glucosidase II (Gls II), which results in a high mannose-type $Man_9GlcNAc_2$ oligosaccharide intermediate. (B) Immunogold detection of glucosidase II in the rough endoplasmic reticulum. Gold particle labeling (black dots) is preferentially over the lumen of the endoplasmic reticulum cisternae since glucosidase II is a luminal, soluble glycoprotein.

protein folding in the ER *in vivo*, confirming and extending evidence obtained by glucosidase inhibitors (Hammond et al., 1994; Hebert et al., 1995). In general terms, di-glucosylated oligosaccharides generated by glucosidase I appear to represent a glyco-code promoting protein *N*-glycosylation by influencing the oligosaccharyltransferase-mediated reaction, whereas mono-glucosylated oligosaccharides represent a glyco-code promoting protein folding through the calnexin/calreticulin cycle.

Mono-glucosylated oligosaccharides and the calnexin/calreticulin cycle

Through the seminal work of Helenius and coworkers, the existence of a folding cycle for mono-glucosylated glycoproteins was elucidated (Hammond and Helenius, 1994a; 1994b; Hammond et al., 1994). Like many chaperones present in the rER that shelter glycoproteins to prevent their aggregation and assist them in folding, the calnexin/calreticulin cycle provides an environment for productive protein folding (Caramelo and Parodi, 2008).

Calnexin and calreticulin are highly related legume lectins with calnexin being a type I membrane protein and calreticulin being a soluble luminal ER protein (Bergeron et al., 1994; Schrag et al., 2003). Both are retained in the ER through specific localization signals at their C-termini and have been found to form complexes with ERp57, a thiol-disulfide oxidoreductase. The carbohydrate-binding specificity of calnexin and calreticulin appears to be identical, both require the innermost α1, 3-linked glucose residue present on the branch A of the oligosaccharide (Fig. 4) (Kapoor et al., 2004; Patil et al., 2000; Schrag et al., 2001). However, calnexin and calreticulin appear to interact with different glycoproteins and furthermore, calnexin seems to be more important as an assistant for proteins to fold (Hebert et al., 1997; Molinari et al., 2004; Pieren et al., 2005). Regardless, the first trimming reaction by glucosidase II resulting in the $Glc_1Man_9GlcNAc_2$ oligosaccharide permits entry of the glycoprotein in the calnexin/calreticulin cycle (Fig. 4). The ensuing lectin-carbohydrate interaction acts in a chaperone-like fashion by sequestering the mono-glucosylated glycoproteins. It seems that all newly synthesized glycoproteins associate with calnexin or calreticulin. Exit from the calnexin/calreticulin cycle requires glucosidase II as well, which removes the innermost glucose residue.

The fate of de-glucosylated glycoproteins after their exit from the calnexin/calreticulin cycle strongly depends on their conformation. Those with a native conformation can exit the ER, whereas non-native conformers will enter another calnexin/calreticulin cycle. Re-entry in the calnexin/calreticulin cycle is granted by UDP-glucose:glycoprotein glucosyltransferase (GT) (Parodi et al., 1983; Trombetta and Parodi, 2003). The GT is basically ubiquitous and therefore found in *S. pompe*, *Drosophila melanogaster* and mammalian cells. However, it should be noted that it is not detectable in *S. cerevisiae* (Table 1). Thus, in *S. cerevisiae* non-native conformers cannot enter another

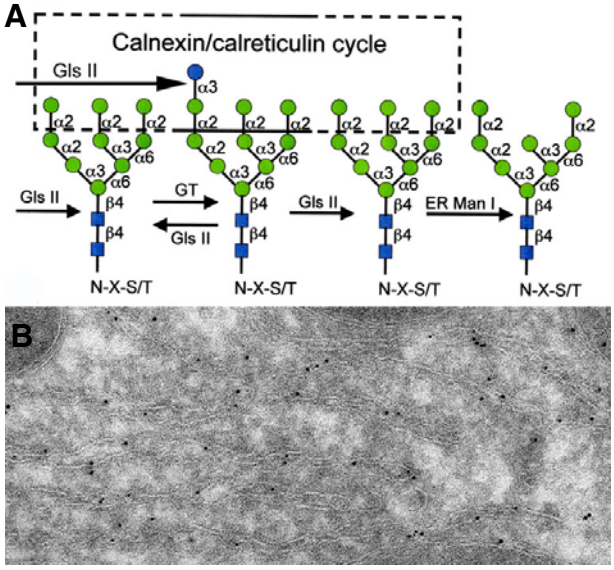

Fig. 4. (A) The calnexin/calreticulin cycle. On the entry side (left part of the box), proteins with the $Glc_1Man_9GlcNAc_2$ oligosaccharide generated by glucosidase II (Gls II) or by glucosyltransferase (GT) are a substrate for calnexin or calreticulin (solid lines of the boxed field). After de-glucosylation by Gls II and release from calnexin or calreticulin, for not correctly folded glycoproteins further re-glucosylation by GT and re-entry in the calnexin/calreticulin cycle may occur (left part of the box) or definite exit (right part of box) followed by mannose trimming by ER mannosidase I (ER Man I) to yield a $Man_8GlcNAc_2$ isomer B. (B) Immunogold detection of glucosyltransferase in the rough endoplasmic reticulum of a rat liver hepatocyte.

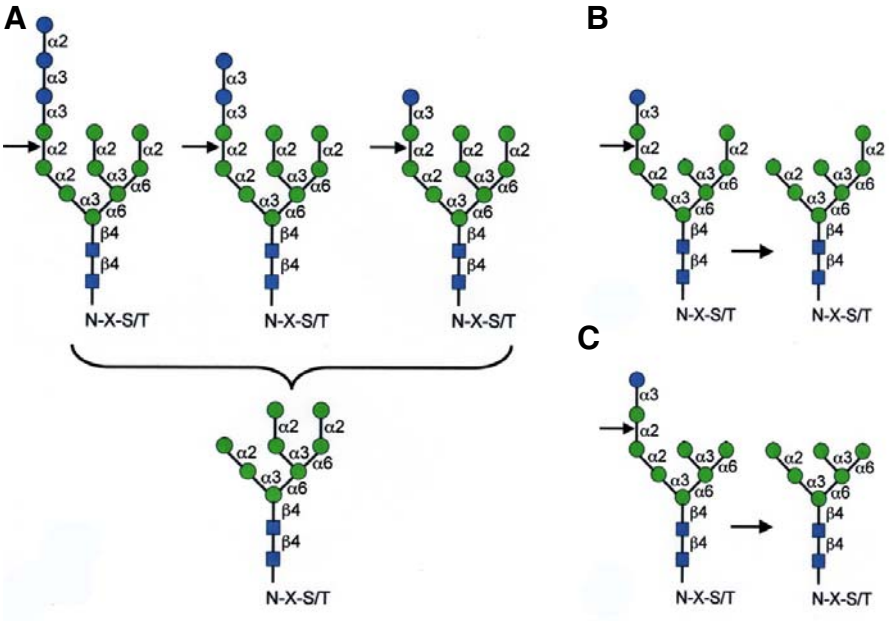

Fig. 5. Various glucosylated oligosaccharides are substrate for Golgi endomannosidase. (A) Endomannosidase has a substrate specificity for tri-glucosylated oligosaccharides like glucosedase I (left) or for di- and mono-glucosylated oligosaccharides like glucosidase II (center and right). However, unlike glucosidase I or II, the product of its action is a $Man_8GlcNAc_2$ isomer A oligosaccharide. This oligosaccharide is no acceptor substrate for GT. (B, C) Endomannosidase also has specificity for mannose-trimmed, mono-glucosylated oligosaccharides yielding a $Man_7GlcNAc_2$ (B) or a $Man_6GlcNAc_2$ oligosaccharide (C).

calnexin cycle, but will be processed by ER mannosidase I (see below).

GT is a soluble luminal glycoprotein, which is present throughout the smooth and rER and concentrated in the pre-Golgi intermediates (Zuber et al., 2001). Notably, GT acts in a two-fold manner, first as a folding sensor and second as a glycosyltransferase (Trombetta and Parodi, 2003). It will bind to non-native conformers most probably through interaction with exposed hydrophobic amino acid patches. This is followed by the re-glucosylation reaction and re-entry in the calnexin/calreticulin cycle (Fig. 4). Glucosylation by GT is transient since the glucose residue will be removed by glucosidase II for exit from the calnexin/calreticulin cycle. Re-glucosylation by GT occurs most efficiently when all nine mannoses are present and is less efficient for glycoproteins with $Man_8GlcNAc_2$ and $Man_7GlcNAc_2$ oligosaccharides (Sousa et al., 1992). It is assumed that some mannose trimming will occur between repeated calnexin/calreticulin cycles, which may affect subsequent processing by GT and glucosidase II. However, some controversy exists regarding the rate of re-glucosylation by GT (Grinna and Robbins, 1980) and of de-glucosylation by glucosidase II (Ermonval et al., 2001) of differentially mannose-trimmed oligosaccharides. Notwithstanding, various lines of evidence indicate the importance of mannosidase activities in the ER for quality control of protein folding and their role in delaying and eventually preventing re-entry of not correctly folded glycoproteins in the calnexin/calreticulin cycle.

Mannose trimming and a role for degradation of aberrant glycoproteins

Following glucose trimming and transient re-glucosylation, trimming may ensue by mannosidase(s) present in the ER (Herscovics, 1999; Moremen et al., 1994). Both, the folding state of a glycoprotein and the extent to which mannose residues have been removed seem to be important factors for the ER-associated degradation (ERAD) of not correctly folded glycoproteins.

ER mannosidase I is an α1, 2-mannosidase with a type II membrane protein topology, belongs to the glycosyl hydrolase family 47 and exists from yeast (with the exception of *S. pompe*) to mammalian cells (Gonzalez et al., 1999; Tremblay and Herscovics, 1999) (Table 1). The substrate for ER mannosidase I is the $Man_9GlcNAc_2$ oligosaccharide generated by the combined action of glucosidase I and II. ER mannosidase I cleaves a single α1, 2 mannose residue to yield the $Man_8GlcNAc_2$ isomer B oligosaccharide. Since *S. pompe* lacks ER mannosidase I (Table 1), trimming in this yeast does not occur further than the $Man_9GlcNAc_2$ oligosaccharide, but this does not affect reglucosylation by GT. Although it is ample demonstrated that mannose trimming occurs beyond the $Man_8GlcNAc_2$ oligosaccharide derived from ER mannosidase I, it is much less obvious through which activities this occurs. The possible contributions of other mannosidase activities such as endo α1, 2 mannosidase (Fig. 5), which is present in the pre-Golgi intermediates and the *cis*/medial Golgi apparatus (Lubas and Spiro, 1987; Zuber et al., 2000) or mannosidases IA, IB and IC, which reside in the *cis* Golgi apparatus (Lal et al., 1994; 1998; Tempel et al., 2004; Tremblay and Herscovics, 2000; Zuber and Roth, 2009) remain to be elucidated in more detail. It is known that non-native conformers may escape the ER and will be subsequently retrieved, and that endo α1, 2 mannosidase acts on native and not correctly folded glycoprotein (Torossi et al., 2006). Furthermore, overexpression of ER mannosidase I has been shown to result in increased mannose trimming to yield $Man_{5\text{-}6}GlcNAc_2$ oligosaccharides (Avezov et al., 2008; Hosokawa et al., 2003). It has been stressed that this condition may not bear a close relationship to the physiological condition in cells and that ER mannosidase I may not be as specific as generally assumed (Herscovics et al., 2002). Noteworthy, ER mannosidase I together with ERAD substrates has been shown to accumulate in a pericentriolar location and it was proposed that this local concentration of mannosidase would permit additional mannose trimming (Avezov et al., 2008). Since a variety of different structures including endosomes, lysosomes and aggresomes are preferentially localized near the centriole (Pavelka and Roth, 2010), it will be important to identify this structure by electron microscopy. Other conditions were found to result in further mannose trimming. Overexpression of EDEM1/ Htm1 protein (see below) in mammalian cells (Hosokawa et al., 2010; Olivari et al., 2006) or in yeast (Clerc et al., 2009), or of EDEM3 (Hirao et al.,

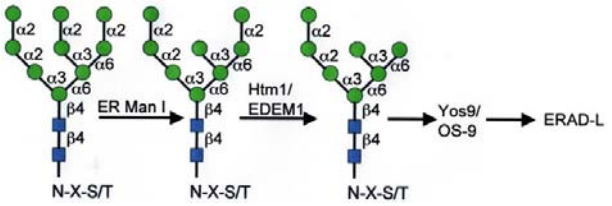

Fig. 6. Current view of mannose trimming of not correctly folded glycoproteins to generate a $Man_7GlcNAc_2$ oligosaccharide with a terminal α1, 6 mannose residue for binding by Yos9/OS-9 lectin. See text for details.

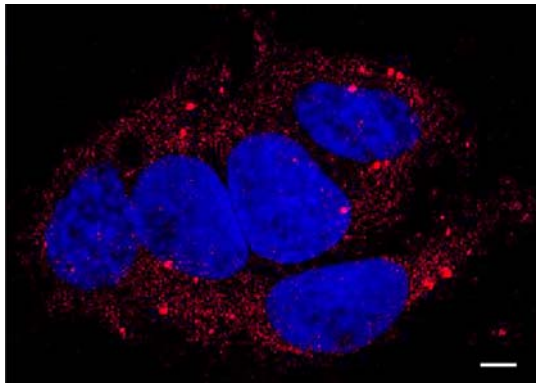

Fig. 7. EDEM1 detection by confocal immunofluorescence in a small cluster of human hepatoma HepG2 cells. In addition to a fine punctate pattern throughout the cytoplasm, some larger spots can be seen, which represent EDEM1 in autophagosomes. Bar: 5 μm.

2006) has been shown to result in additional mannose trimming to yield $Man_7GlcNAc_2$ oligosaccharides. This involved the removal of a mannose residue of the C branch (Clerc et al., 2009; Hosokawa et al., 2010). At least for EDEM1 of mammalian cells, it must be taken into consideration that the subcellular distribution of overexpressed as compared to endogenous EDEM1 is different (Zuber et al., 2007). Overexpressed EDEM1, in striking contrast to endogenous EDEM1, is distributed throughout the ER and this may result in effects normally not occurring (Le Fourn et al., 2009).

To further complicate the situation, an additional mannosidase, ER mannosidase II, probably exists in mammalian cells. This mannosidase also trims the $Man_9GlcNAc_2$ oligosaccharide, but more excessively to a $Man_{5-6}GlcNAc_2$ oligosaccharide (Bischoff and Kornfeld, 1983; Bischoff et al., 1986; Weng and Spiro, 1996). Such additional mannose trimming to $Man_{5-6}GlcNAc_2$ has been shown to be relevant for ERAD (Ermonval et al., 2001; Foulquier et al., 2004; Frenkel et al., 2003; Kitzmuller et al., 2003). As mentioned above, it appears also to negatively affect the rate of re-glucosylation by GT required for re-entry in the calnexin/calreticulin cycle.

In summary, in *S. cerevisiae* ER mannosidase I and its product of action $Man_8GlcNAc_2$ isomer B, in the absence of GT, appear to be sufficient for routing of non-native conformers to ERAD. In mammalian cells additional mannosidase activities, and the $Man_{7-5}GlcNAc_2$ oligosaccharides generated by them, appear to be a requirement. This altogether illustrates the complexity of mannose trimming involved in ERAD. However, there is additional intricacy. As already mentioned, an endomannosidase exists in higher eukaryotes, which not only acts on monoglucosylated but additionally on di- and tri-glucosylated oligosaccharides (Fig. 5) and cleaves the glycosidic bond between the glucose-substituted mannose and the remaining oligosaccharide of the A branch (Spiro, 2000; Spiro et al., 1997). This prevents re-glucosylation by GT. Endomannosidase, however, is located in the pre-Golgi intermediates and in the *cis*/medial Golgi apparatus and is thought to process glucosylated oligosaccharides of glycoproteins that have escaped processing by glucosidases (Zuber et al., 2000). If retrieved to the ER, not correctly folded glycoproteins processed by endomannosidase will become ERAD substrate. On the other hand, they are also substrate for Golgi mannosidases while present in this organelle. Not only the potential role of endomannosidase requires more studies, it will be worthy to analyze the *cis* Golgi mannosidases I A-C as well.

Dislocation of aberrant glycoproteins from the ER to the cytoplasm: EDEM1 and OS-9

The initial finding of the importance of the $Man_8GlcNAc_2$ isomer B on misfolded carboxypeptidease Y in *S. cerevisiae* (Jakob et al., 1998a) and misfolded alpha 1-antitrypsin in mammalian cells (Liu et al., 1999) for subsequent ERAD initiated the search for proteins likely to be involved in the recognition and binding of this oligosaccharide structure. In mammals and yeast, mannosidase homologues were discovered that seemed to fulfill the expectation. These are EDEM1 in mammalian cells (Hosokawa et al., 2001; Mast et al., 2005) and its yeast homologues Htm1p or Mnl1p (Jakob et al., 2001; Nakatsukasa et al., 2001). Two additional EDEM proteins, EDEM2 and EDEM3, were discovered subsequently (Hirao et al., 2006; Mast et al., 2005; Olivari et al., 2005). EDEM proteins are soluble glycoproteins, which belong to the class I α-mannosidases because of a glycosyl hydrolase family 47 (mannosidase) domain in their N-terminal domain (Hirao et al., 2006; Mast et al., 2005; Olivari et al., 2005). EDEM1, 2 and 3, however, differ in the structure of their C- and N-terminal extensions.

Overexpression of EDEM1 resulted in accelerated release of misfolded glycoproteins from the calnexin/calreticulin cycle and their subsequent degradation, whereas its down-regulation had the opposite effect (Molinari et al., 2003; Oda et al., 2003). When overexpressed, EDEM2 showed the same effect on ERAD of misfolded glycoproteins. Likewise, EDEM3 was shown to accelerate degradation of misfolded alpha 1-antitrypsin, but in addition it has also α1, 2 mannosidase activity (Hirao et al., 2006). Therefore, its mechanism for ERAD of misfolded glycoproteins appears to be different from the two other EDEM proteins. In addition to EDEM1/Htm1, OS-9/Yos9 selectively binds to non-native conformers of glycoproteins and routes them in the ERAD pathway (Bhamidipati et al., 2005; Buschhorn et al., 2004; Kim et al., 2005; Szathmary et al., 2005). OS-9/Yos9 is a glycoprotein that contains a conserved mannose 6-phosphate receptor homology (MRH) domain. OS-9 as compared to the yeast ortholog does not contain the potential ER retention sequence KDEL (Christianson et al., 2008). The MRH domain of OS-9/Yos9 is involved in oligosaccharide recognition based on observations of point mutations targeting conserved residues.

Various mechanisms, which involve distinct ubiquitin-ligase complexes, have been proposed to function in the management of ERAD substrates depending whether the misfolded domain is luminal (ERAD-L), cytosolic (ERAD-C) or intramembrane (ERAD-M) (Carvalho et al., 2006; Denic et al., 2006). Both EDEM1/Htm1 and OS-9/Yos9 appear to be involved in the ERAD-L pathway. However, until recently, their roles were not well characterized. EDEM1/Htm1 was originally proposed to be a lectin-like protein and to interact with the $Man_8GlcNAc_2$ isomer

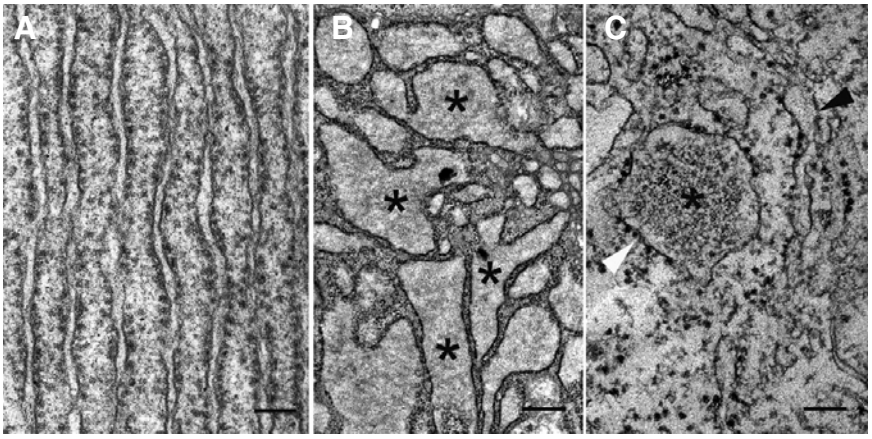

Fig. 8. (A) The lumen of the cisternae of rough endoplasmic reticulum in secretory cells is usually narrow. (B) In contrast, the lumen of the rough endoplasmic reticulum of a Mott cell is greatly distended (asterisks) as it contains large amounts of secretion-incompetent immunoglobulins. (C) A Russel body (asterisk) is a local distension of a rough ER cisterna due to luminal protein aggregates, which occur as an electron dense amorphous material. The white arrowhead points to ribosomes at the Russel body membrane and the black arrowhead to a normal appearing rough ER cisterna Bar: 0.1 μm (A, C); 0.2 μm (B).

B, but such an interaction was never directly demonstrated. Although EDEM1 binds selectively to not correctly folded glycoproteins such as the Hong Kong variant of alpha 1-antitrypsin (Cormier et al., 2009; Hosokawa et al., 2001; 2003), it was recently shown that this interaction occurred oligosaccharide-independent (Cormier et al., 2009). However, the mannosidase-like domain of EDEM1 was found to be important for an oligosaccharide-dependent interaction with SEL1 and that this interaction was targeting misfolded alpha 1-antitrypsin for ERAD (Cormier et al., 2009). A previous work (Christianson et al., 2008) demonstrated that OS-9 delivered mutant alpha 1-antitrypsin to SEL1 and Hrd1 for ERAD. At the same time, the oligosaccharide-binding specificity of Yos9 was determined to be a mannose$_7$-trimmed oligosaccharide with an exposed α1, 6 mannose (Quan et al., 2008). Such a substrate specificity was also reported for OS-9 (Hosokawa et al., 2009). Since the generation of a $Man_7GlcNAc_2$ oligosaccharide requires a first trimming by ER mannosidase I and the trimming of another mannose residue at the C branch to expose the α1, 6 mannose residue, Htm1 was suggested to act upstream to generate the specific $Man_7GlcNAc_2$ isomer (Quan et al., 2008). Although no mannosidase activity for EDEM1/Htm1 (and for EDEM2) could be detected in earlier studies, two recent works reported mannose trimming activity for overexpressed Htm1 (Clerc et al., 2009) and for overexpressed EDEM1 (Hosokawa et al., 2010). Although as discussed above, the specific $Man_7GlcNAc_2$ oligosaccharide structure may be generated through the action of various enzymes, for the time being the mechanism for glycan-dependent dislocation and degradation of a misfolded glycoprotein by ERAD-L can be summarized as follows (Fig. 6). After exit from the calnexin/calreticulin cycle, ER mannosidase I processes the oligosaccharide(s) on not correctly folded glycoproteins to $Man_8GlcNAc_2$, which in turn is converted by EDEM1/Htm1 to $Man_7GlcNAc_2$ to provide the oligosaccharide for interaction with OS-9/Yos9. The latter would provide the link to the Hrd1/Hrd3 ubiquitin ligase complex. Although the current model considers exclusively the $Man_7GlcNAc_2$ oligosaccharide and yeast, it was pointed out that the highest affinity of Yos9 was for $Man_5GlcNAc_2$ oligosaccharides (Quan et al., 2008). This clearly indicates that more aspects of ERAD-L wait to be revealed. In addition, the nature and structure of the membrane dislocation machinery needs to be elucidated in further detail.

Subcellular topography and turnover of quality control machinery proteins

Glucosidase II, GT and EDEM1 as well as calreticulin have been localized by high resolution immunogold electron microscopy. Furthermore, for myc-tagged ER mannosidase I (Gonzalez et al., 1999) and endogenous OS-9 as well as Hrd1 (Christianson et al., 2008) confocal immunofluorescene data are available indicating an ER distribution and overlap with calreticulin and KDEL, respectively. Both, glucosidase II (Lucocq et al., 1986; Zuber et al., 2000; 2001) and GT (Zuber et al., 2001) showed overlapping distributions in the rER including nuclear envelope (Figs. 3 and 4) and in the smooth ER as well as additionally in pre-Golgi intermediates. The confocal immunofluorescence pattern for EDEM1 (Fig. 7) did not match with that of calreticulin and by high resolution immunogold labeling, endogenous EDEM1 was found mainly outside the rER in smooth vesicles and restricted to small portions of a few rER cisternae (Zuber et al., 2007). The EDEM1-positive vesicles originated outside of the ER exit sites and were not labeled for the COPII protein Sec23 nor were they positive for ERGIC-53. These data indicate the existence of an ER vesicular exit pathway in addition to the canonical COPII pathway, although its details remain to be worked out. Noteworthy, the EDEM1 subcellular distribution did not change in cells overexpressing the Hong Kong variant of alpha 1-antitrypsin (Zuber et al., 2007). However, expression of FLAG-tagged EDEM1 in various cell types resulted in its predominant ER localization (Zuber et al., 2007). Such an altered subcellular distribution due to overexpressing EDEM1 resulted in a block of cell division and caused apoptosis (Le Fourn et al., 2009).

Both, endogenous glucosidase II (Strous et al., 1987) and HA-tagged ER mannosidase I (Hosokawa et al., 2003) were found to turn over rapidly. The degradation of glucosidase II seems to involve autophagy (Lucocq et al., 1986). The degradative pathway of ER mannosidase I is less clear, but does not involve proteasomes (Hosokawa et al., 2003; Wu et al., 2007). Endogenous EDEM1 is also a short-lived glycoprotein and becomes degraded by autophagy (Le Fourn et al., 2009). Together, this indicates that the expression level of these quality control machinery proteins is tightly controlled. Clearly, the details of the degradative pathways and their functional significance deserve further analysis.

Morphology of cells synthesizing misfolded proteins

The presence of not correctly folded secretory or membrane proteins in the secretory pathway of cells results in the upregulation of the unfolded protein response (UPR) (Bernales et al., 2006; Jonikas et al., 2009; Schroder and Kaufman, 2005). This causes an extensive transcriptional response, which may result in successful cellular adaptation or in apoptosis. Under physiological conditions, a high percentage of *de novo* synthesized

proteins may not achieve a native conformation and will be degraded and recycled as waste products. Diverse proteins may not fold correctly because of disease-causing mutations and will be retained in the ER because of the quality control. However, this will not automatically result in ultrastructural changes since they not only depend on the amounts of misfolded proteins and the efficiency of the degradative pathways such as the ubiquitin-proteasome system but also on additional factors such as the propensity of misfolded glycoproteins to form self-aggregates or to interact with proteins in addition to chaperones. In addition, since most disorders caused by protein misfolding are chronic diseases, ultrastructural changes may be observed only late. Lysosomal enzymes, although abundant in lysosomes, are not major proteins among the *de novo* synthesized proteins in the ER. The presence of mutant lysosomal enzymes such as of mutant α-galactosidase A causing Fabry disease or of mutant glucocerebrosidase resulting in Gaucher's disease does not result in structural abnormalities of the ER such as dilation of its lumen (Pavelka and Roth, 2010; Yam et al., 2005; 2006). Likewise, ER-retained mutant aquaporin 1, a multispanning membrane protein, is efficiently degraded by proteasomes without causing ER structural changes (Hirano et al., 2003). In contrast, disease-causing mutations of thyroglobulin, a secretory glycoprotein, results in greatly distended ER (Kim and Arvan, 1998; Kim et al., 2000) and similar ultrastructural changes are caused by secretion-incompetent immunoglobulins in Mott cells (Alanen et al., 1985) (Fig. 8). Local distension of the rER, so called Russel bodies, are due to luminal protein aggregates such as mutant immunoglobulins (Alanen et al., 1985; Kopito and Sitia, 2000; Mattioli et al., 2006), the Z variant of alpha 1-antitrypsin (Hidvegi et al., 2005; Lomas et al., 1992; 2004), or mutant myocilin (Yam et al., 2007). As discussed above, pre-Golgi intermediates may be involved in quality control and become enlarged due to accumulation of mutant deltaF508 chloride channels (Gilbert et al., 1998), misfolded MHC I protein (Hsu et al., 1991; Raposo et al., 1995), and misfolded proinsulin (Fan et al., 2007; Zuber et al., 2004).

CONCLUSIONS AND PERSPECTIVES

Quality control of protein folding provides an example of the close functional relationship between *N*-glycosylation of proteins and protein folding in a single organelle, the ER. It also provides a prime example for translational research since much of the basic knowledge on protein folding bears a direct relation to the elucidation of the pathogenesis of human diseases and potentially to the development of new forms of therapy. Although many of the molecular details are conserved between yeast and mammalian cells, profound differences exist which necessitate further studies on the molecular composition of the quality control machinery. It also should be pointed out that quality control of folding of secretory and membrane proteins is an inherently leaky process not only in yeast but also in mammalian cells. In mammalian cells, this may be advantageous since many of the misfolded proteins have some residual or even full biological activity and thus, the severity of a given protein folding disorder can vary. Despite all progress, particularly in yeast, there is still a lack of knowledge about the fine details of the dislocation from the ER lumen to the cytosol of the various misfolded luminal proteins and about the architecture of the presumptive hydrophilic dislocator.

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